USO DE CARACTERÍSTICAS BIOQUÍMICAS-FISIOLÓGICAS, CELULARES E SUB-CELULARES DE *BETULA PENDULA* COMO MARCADORES DE GERMINAÇÃO DE SEMENTE E MONITORAMENTO DA POLUIÇÃO DO TERRITÓRIO**USE OF PHYSIOLOGICAL-BIOCHEMICAL, CELLULAR AND SUB-CELLAR CHARACTERISTICS OF *BETULA PENDULA* AS MARKERS OF SEED GERMINATION AND MONITORING TERRITORY POLLUTION****ИСПОЛЬЗОВАНИЕ ФИЗИОЛОГО-БИОХИМИЧЕСКИХ, КЛЕТОЧНЫХ И СУБКЛЕТОЧНЫХ ХАРАКТЕРИСТИК *BETULA PENDULA* КАК МАРКЕРОВ ВСХОЖЕСТИ СЕМЯН И МОНИТОРИНГА ЗАГРЯЗНЕНИЯ ТЕРРИТОРИЙ**VOSTRIKOVA, Tatyana V. ^{1*}; ZEMLYANUKHINA, Olga A. ¹; KALAEV, Vladislav N. ¹¹ Botanical Garden of Voronezh State University, Botanical Garden, 1, Voronezh, 394068 Russia

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Received 12 June 2019; received in revised form 01 August 2019; accepted 12 August 2019

RESUMO

A quantidade de índices citogenéticos de proteínas solúveis totais, capacidade de germinação na progênie de sementes de *Betula pendula* em áreas com diferentes níveis de poluição antropogênica foi estudada. A progênie de sementes de *Betula pendula* possui capacidade de germinação aumentada e proteína total em áreas com baixos níveis de poluição quando comparadas ao grupo controle (sementes coletadas em território ecologicamente limpo) e aos mesmos parâmetros para as áreas com altos níveis de pressão antropogênica. Uma alta correlação positiva foi estabelecida entre a germinação de sementes e a quantidade de proteína solúvel total. O parâmetro “quantidade de proteína total” é caracterizado como marcador de germinação de sementes, o que determina a possibilidade de sua germinação. Parâmetros citogenéticos (atividade mitótica, razão percentual do número de células nos estágios da mitose, nível de patologias da mitose, nível de células com nucléolo persistente na fase de mitose anafásica) de mudas de bétula foram estudados como marcadores de poluição antrópica de territórios. Relações entre características citogenéticas e bioquímicas foram encontradas. O impacto do ambiente, causado principalmente pela poluição por metais pesados, em árvores de bétulas e suas progênies, pode ser definido como hormese por índices bioquímicos e citogenéticos. Um estudo abrangente de parâmetros citogenéticos e bioquímicos pode permitir uma avaliação rápida e adequada da qualidade de sementes de origem desconhecida que é especialmente importante para a indústria de plantas e florestas, pois permite selecionar material de plantio estável e coletar material para fins farmacognósticos em áreas com pressão ecológica diferente. Determinar os índices citogenéticos e a quantidade de proteína total permite prever as capacidades de germinação e capacidade de germinação das sementes da bétula.

Palavras-chave: progênie de sementes, capacidade de germinação, índices citogenéticos, poluição antropogênica, atividade mitótica.

ABSTRACT

The amount of total soluble protein cytogenetic indices, germination ability in the seed progeny of *Betula pendula* in areas with different levels of anthropogenic pollution was studied. The seed progeny of *Betula pendula* has an increased germination ability and total protein in areas with low pollution levels as compared to the control group (seeds collected in the ecologically clean territory) and to the same parameters for the areas with high levels of anthropogenic pressure. A high positive correlation was established between seed germination and the amount of total soluble protein. The parameter “amount of total protein” is characterized as a marker of seed germination, which determines the possibility of their germination. Cytogenetic parameters (mitotic activity, percentage ratio of cell number at mitosis stages, level of mitosis pathologies, level of cells with persistent nucleoli at metaphase - anaphase mitosis stage) of seed seedlings of weeping birch were studied as marker signs of anthropogenic pollution of territories. Relations between cytogenetic and biochemical characteristics were found. The impact of the environment, caused mainly by heavy metal pollution, on birch

trees and their seed progeny, can be defined as hormesis by biochemical and cytogenetic indices. A comprehensive study of cytogenetic and biochemical parameters can allow a quick and adequate evaluation of the seed quality of unknown origin which is especially important for plant industry and forestry as it allows selecting stable planting material and for the collection of material for pharmacognostic purposes in areas with different ecological pressure. Determining cytogenetic indices and the amount of total protein allows forecasting the germination capacities and germination abilities of the seeds of the weeping birch.

Keywords: seed progeny, germination ability, cytogenetic indices, anthropogenic pollution, mitotic activity.

АННОТАЦИЯ

Исследованы цитогенетические показатели, всхожесть семян и количество общего белка у семенного потомства березы повислой в районах г. Воронежа с различной антропогенной нагрузкой. У семенного потомства *Betula pendula* в слабозагрязненном районе увеличивается всхожесть и количество общего растворимого белка в сравнении с контролем (семенами, собранными на экологически чистой территории) и с данными параметрами в районе с сильной антропогенной нагрузкой. Установлена высокая положительная корреляция между всхожестью семян и количеством общего растворимого белка. Параметр «количество общего белка» характеризуется как маркер всхожести семян, определяющий возможность их прорастания. Исследованы цитогенетические параметры (митотическая активность, процентное соотношение числа клеток на стадиях митоза, уровень патологий митоза, уровень клеток с остаточными ядрышками на стадии метафазы – анафазы митоза) семенного потомства березы повислой как маркерные признаки антропогенного загрязнения территорий. Найдены связи между цитогенетическими и биохимическими характеристиками. Воздействие окружающей среды, вызванное преимущественно загрязнением тяжелыми металлами, на деревья березы повислой и их семенное потомство по биохимическим и цитогенетическим показателям можно определить как гормезис. С помощью комплексного исследования цитогенетических и биохимических параметров возможна быстрая и адекватная оценка качества семян неизвестного происхождения, имеющая особое значение в растениеводстве, лесоводстве для отбора устойчивого растительного материала, а также при сборе сырья для фармакогнозии на территориях с различной экологической напряженностью. Определение цитогенетических показателей и количества общего белка позволяет прогнозировать возможности всхожести и прорастания семян березы повислой.

Ключевые слова: семенное потомство, всхожесть семян цитогенетические параметры, антропогенное загрязнение, митотическая активность.

1. INTRODUCTION

Morphological (Opalko and Opalko, 2015), geobotanical (Kuzemko, 2016), cytogenetic (Kalaev *et al.*, 2006; Baranova and Kalaev, 2017; Yakymchuk, 2015) studies, and modeling of plants' reactions to extreme factors in cultivated cenosis (Bome *et al.*, 2015) are used to assess the environmental situation. The modeling and extrapolation of the results from plants to humans have been used over recent years to forecast the environmental effect on the health of the population (Baranova and Kalaev, 2017; Chopikashvili *et al.*, 2016; Butorina *et al.*, 2002).

Weeping birch (*Betula pendula* Roth) is a prevalent woody plant known for its medicinal properties and classified among main forest-forming species in European Russia. This species is a valuable material for the timber, wood chemical, pulp and paper, and other

industries. It also has a high decorative value.

Due to the biologically active substances, weeping birch has a higher or lower phytoncidal activity in different periods of the growing season (Vetchinnikova, 2004; Bukharina, 2011) and is widely used in sustainable building (Neverova, 2004; Bukharina *et al.*, 2012; Erofeeva and Naumova, 2012). The phytoncidal properties are crucial for planting in urban areas affected by technogenic pollution. Therefore, to fulfill this function, it is highly important to select plants resistant to anthropogenic pressure. Weeping birch is being actively studied in the area of anatomy (Makhnev *et al.*, 2012; Migalina, 2011), morphology (Bukharina *et al.*, 2012; Erofeeva and Naumova, 2012; Erofeeva, 2015), biochemistry (Vetchinnikova, 2004; Bukharina, 2011; Neverova, 2004; Bukharina *et al.*, 2012; Erofeeva and Naumova, 2012), and cytogenetics (Kalaev *et al.*, 2006; Baranova and Kalaev, 2017; Chopikashvili *et al.*, 2016; Butorina and

Kormilitsyna, 2001; Vostrikova, 2007; Kalaev *et al.*, 2010). As *Betula pendula* is a perennial plant, it may experience lasting impact from environmental mutagens, accumulate doses of mutagens, and be used in cytogenetic studies, being one of the most sensitive species to bio-indicators (Kalaev *et al.*, 2006; Baranova and Kalaev, 2017; Bukharina, 2011; Erofeeva, 2015; Butorina and Kormilitsyna, 2001; Vostrikova, 2007; Kalaev *et al.*, 2010).

The study of the changing amount and composition of soluble protein in seeds is essential as it is related to their quality and can be used to find the best storage conditions and to study the agronomic features of woody plants. Earlier research showed an increase in highly soluble protein in the cytoplasm during the first hours of pollen tube growth (Ilchenko, 1990). The study of the cytochemical properties of chromatin in germinating seeds in relation to their economic important traits has been conducted (Trojan *et al.*, 1990). However, this study was primarily dedicated to agricultural plants: soy (Korsunova *et al.*, 2000), rape (Murtazaliyeva and Abakargadzhieva, 2011), sunflower (Minakova *et al.*, 1996), etc. Data about the changing content of total protein in the seeds of woody plants is scarce: *Acer negundo* L., *A. pseudoplatanus* L., *Fraxinus lanceolata* Borkh. (Yusypiva and Koval, 2011). The correlation between the speed of the chromatin activation, germinating energy, and the seed germination ability was established, and the special nature of these processes depending on the species and variety was found, which was demonstrated using various varieties of peas, wheat, and corn (Trojan *et al.*, 1990). Earlier research did not show a correlation between the seed germination ability and the amount of total soluble protein in woody plants.

Due to the deteriorating environmental situation, the effects of the anthropogenic pressure on the living organisms have been studied, including those on the cellular and molecular level. The change in protection system indices (peroxidase activity, the content of total protein and the aggregate number of sulfhydryl groups of protective peptides) in levels of weeping birch and little-leaf linden under the effects of auto pollution was studied earlier in Nizhny Novgorod (Erofeeva, 2016). However, there was no comprehensive research of cytogenetic parameters, germination abilities, and the amount of total protein in seed progeny, as well as an attempt to find a correlation between them. A comprehensive study of cytogenetic and biochemical indicators will allow a quick and

adequate evaluation of the seed quality, especially for the plants whose seeds have a short storage life.

The purpose of this paper is to study the amount of total soluble protein, germination abilities, and cytogenetic indicators for the seed progeny of *Betula pendula* collected in the ecologically clean territory and under anthropogenic pollution of different level.

2. MATERIALS AND METHODS

The research was conducted in two districts of Voronezh with different levels of anthropogenic pollution: Central district, in Platonov street (area with a low pollution level) and Levoberezhny district on Leninsky avenue (area with a high pollution level). As a reference (ecologically "clean") area, we used the B.M. Kozo-Polyansky Botanical Garden of Voronezh State University (VSU), where environmental pollution is within permissible values (Kurulap *et al.*, 2010, 2012).

Seeds collected from 15 (5 in the ecologically "clean" and 5 in each of the polluted areas) trees of weeping birch (*Betula pendula* Roth) were used as plant material for the research. Seeds were germinated, and their germination abilities were assessed in accordance with the requirements of GOST standard 13056.6-97 (1997). The seeds were germinated at a constant temperature +25°C in 3 repeats 100 seeds each. The laboratory seed germination was determined as a correlation between the number of germinated seeds to the total number of seeds and was expressed in %, as recommended by the requirements of GOST standard 13056.6-97 (1997). In order to obtain protein preparations, a weighed portion of one seed (10 seeds from every tree were studied) was ground with glass sand in 0.1 M tris-NS1 buffer at pH 7.5, and centrifuged for 10 min at 20,000 g, 4 °C, in a CM50 ELMI centrifuge (Latvia). The total soluble protein content was measured in the supernatant following a standard method (Bradford, 1976). Bovine serum albumin (Sigma) was used as a standard.

When the roots reached a length of 0.5 to 1 cm, they were fixed at 9 am o'clock in acetic alcohol: the 3:1 mixture of 96% ethanol and glacial acetic acid, when the mitotic activity of *B. pendula* is in the peak (Vostrikova and Butorina, 2006). The roots were immersed in acetic alcohol during 24 hours. After that, the material was kept refrigerated in acetic alcohol at a temperature of 4 °C. The roots stained with hematoxylin. Following the method described

above, the roots of plantlets were used to make continuously squashed micropreparations using Hoyer's medium (Vostrikova and Butorina, 2006).

The preparations were examined using a LABOVAL-4 (Carl Zeiss, Jena) microscope with a total magnification $40\times 1.5\times 10$ and $100\times 1.5\times 10$. The cytogenetic characteristics of 150 plantlets of *B. pendula* (10 plantlets of seeds from each tree) collected in the ecologically "clean" and polluted areas were analyzed. About 500 cells were analyzed in each micropropagation (1 preparation corresponds to 1 plantlet). In total, about 75,000 cells were analyzed.

The micropreparations were made in accordance with the method described above (Vostrikova and Butorina, 2006). They used to analyze the following cytogenetic parameters: mitotic activity by calculating the mitotic index (MI) – the ratio of the quantity of dividing cells to the total number of calculated cells (%), percentage of cells at mitotic stages, the level of mitotic pathologies (the ratio of the number of aberrant cells with mitosis aberrations at the stages of metaphase, anaphase, telophase to the number of dividing cells, %), the spectrum of mitotic pathologies (the percentage of every type of mitotic disturbances out of their total number, %), and the level of cells with persistent nucleoli at the stages of metaphase – anaphase of mitosis (the ratio of the number of cells with persistent nucleoli to the total number of cells at the given stages, %). The classification of pathological mitosis was done according to I.A. Alov (1972). The results were processed statistically using the Stadia 7.0 software package. The procedure of data grouping and processing were described by A. P. Kulaichev (2006). A comparison of samples based on mitotic activity and the number of cells in each mitosis stage was conducted using Student's t-test. A comparison of the samples was performed using the Van der Varden rank X-test, based on the level of pathologies of mitosis, the level of cells with persistent nucleoli, and the amount of total soluble protein, as the distribution of these indicators is nonparametric. The germination abilities of seeds were compared by Z-test for equality of frequencies. To estimate the influence of the "tree" on the amount of protein, a Kruskal–Wallis one-way nonparametric analysis of variance was performed. To calculate the correlation dependence, Spearman's rank correlation coefficient (r_s) was used.

3. RESULTS AND DISCUSSION:

The content of the total soluble protein in seeds of weeping birch differed from tree to tree in the same area (in the same sample), i.e., there were phenotypic differences in the total soluble protein content (Table 1). Furthermore, this assumption was proved by the results of the one-way analysis of variance, which showed the influence of the tree factor in all observed areas ($P<0.01$). The most significant difference is between trees growing in clean and the polluted areas, which might indicate that low stress can lead to synergistic effects that level the index, in clean and polluted environments there is an increase in phenotypic diversity in this characteristic. This is in line with the general idea of the weeping birch as a highly polymorphic species (Popov, 2003).

It has been established that there is a considerable increase in the studied characteristic among trees growing in the area with low pollution as compared to the control area ($P<0.001$) and to the trees in the area with high anthropogenic pollution in Leninsky avenue (near OAO "Voronezhskintezkauchuk") ($P<0.01$, $P<0.001$). The results obtained during this study are consistent with the data presented by other researchers who pointed out that the amount of total protein grows under stress (Chirkova, 2002). For example, there is a strict correlation between factions of soluble protein and polyphenols whose character changes with the increased technogenic pressure (Sidorovich and Getko, 1990). Moreover, stress-induced dehydrin proteins were detected in bud cells of *Betula pendula* in regions with contrastive climates (Tatarinova *et al.*, 2017).

The most substantial amount of protein was found among birch trees from the area with low pollution (see Table 1), which grow in the conditions of low anthropogenic pressure. The following pollutants affect living organisms in Voronezh: SO_2 , NO_2 , and heavy metals of various concentrations depending on how polluted the area is. These areas were ranked (by the level of pollution) in earlier research (Kurolap *et al.*, 2010, 2012; Fedorova and Shunelko, 2003).

The total protein content is considered to indicate the ability of a biosystem to maintain a normal balance between anabolic and catabolic processes (Erofeeva, 2016). It was shown that under stress, degradation of biomolecules predominates (predominance of catabolism over their biosynthesis, i.e., anabolism) due to the

organism's increased energy demands to provide for phenotypic adaptation. Therefore, the total protein content decreases as compared to the control level (Erofeeva, 2016; Garkavi *et al.*, 1990).

According to other authors, anthropogenic aromatic aerosols lead to an increase in the soluble, enzyme enriched protein fraction in leaves of the hardiest plants (Sidorovich and Getko, 1990). According to earlier research by other scientists, under regular impact by SO₂, NO₂, and heavy metals on woody plants, the correlation between albumins and globulins in seeds changes (Yusypiva and Koval, 2011). Two types of physiological and biochemical reaction responses were established. They are based either on conformational reorganizations in macromolecules without affecting the cell's gene regulation, or on adaptive change in biosynthesis.

At the heart of these processes are protein and specific metabolisms, which can be affected by the pollutants of different chemical natures (Sidorovich and Getko, 1990). In the other case, protein metabolism is shifted towards the formation of high molecular proteins with low solubility, which is typical of senescent cells and tissues (Sidorovich and Getko, 1990). Environmental impact on maternal plants of the weeping birch and its seed progeny in the area with low pollution can be described as weak stress associated with moderate concentrations of SO₂ and NO₂ in the air and heavy metals in the soil (Fedorova and Shunelko, 2003). For example, according to E. A. Erofeeva, as a result of 4 seasons of monitoring (2010-2013), only in 2013 there was a monotonic increase in sulfhydryl groups in the leaf and a nonmonotonic decrease in the protein due to increased traffic (Erofeeva, 2016). Other authors also observed a decrease in total protein due to various pollutants (Singh *et al.*, 2013).

This study shows a strong stimulating effect on the amount of total protein in the area with low pollution, which was increased 2.4 times as compared to the control group, and a weak stimulation in the area with high pollution (21% increase in the parameter as compared to the control group). The environmental impact on trees of the weeping birch and its seed progeny in areas with different ecological pressure and content of heavy metals (Kurolap *et al.*, 2010, 2012) can be compared with the influence of microelements. For example, it was noticed that protein content in soy seeds affected by microelements increases by 1.5-5.1%. What is more, its maximum content is accumulated under

the influence of boron, zinc, molybdenum, and combined action of molybdenum and copper (Korsunova *et al.*, 2000).

It was shown that the amount of total soluble protein in each studied tree is associated with the seed germination ability: an increase in the first parameter leads to an increase in the second, and the other way round. It can be proved by high positive correlations between these indices ($r_s = 1$ ($P < 0.01$)). The same way as for the index "amount of total soluble protein", birch trees in the area with low pollution had the highest seed germination ability, which was higher than the control value ($P < 0.01$), which proved an assumption about stimulating the effect of small pollutant doses on seed progeny. A positive correlation between the amount of total protein in seeds and their germination ability indicates that there is a relationship between the intensity of growth processes (happening during seeds germination) and seed germination ability to the protein supply. Therefore, the parameter "amount of total protein" (protein level) can be called a seed germination marker that determines its germination abilities. Evaluation of the amount of total protein will allow to quickly and accurately determine seed germination abilities, which are very important for the plant industry, forestry, and other works that involve plant seeds with different storage lives.

The amount of total protein in plantlets characterizes the metabolic processes that happen in seed progeny after seed germination. This parameter in plantlets of the weeping birch was lower (differences are reliable ($P < 0.001$)) than in the seeds although the trend to its changing in different environmental conditions remained (see Table 1). The amount of total soluble protein in seeds collected in the area with high pollution was lower than for the control group and higher than in the case of low pollution (see Table 1). Presumably, synthetic processes in seed progeny collected in different environments do not differ in a qualitative way but differ in a quantitative way (in intensity) depending on how polluted the area is. To prove this assumption, the cytogenetic characteristics of plantlets should be considered.

This research showed that the amount of total protein (biochemical parameter) is associated with cytogenetic characteristics. Cytogenetic parameters of seed progeny of *Betula pendula* under different ecological conditions are shown in Table 2.

The obtained data shows that *B. pendula*

have a decreased mitotic index in the experiment as compared to the control group (differences are reliable $P < 0.05$; $P < 0.01$; $P < 0.001$) and an increased level of mitotic pathologies and level of cells with persistent nucleoli. What is more, the percentage of mitotic pathologies in the area with high pollution was higher than in the area with low pollution (differences are reliable ($P < 0.01$)). Therefore, the level of mitotic pathologies depends on the level of pollution, which has been mentioned many times by us and other authors (Baranova and Kalaev, 2017; Vostrikova, 2007; Kalaev *et al.*, 2010; Vostrikova and Butorina, 2006).

There is a change in the duration of the mitotic stages due to a significant increase in the number of cells at the prophase stage (differences are reliable ($P < 0.001$)), which is more evident in the area with high pollution (see Table 2). It means that cells are delayed at prophase due to mitotic apparatus disturbances and functioning of the system of checkpoint control of genetic material integrity (Lebedeva *et al.*, 20004). The delay of cells in prophase is associated with the following parameters: "the mitotic index calculated including cells at the prophase stage" and "mitotic index calculated excluding prophase cells" (the delay of cells in prophase is accompanied by an increase in the former parameter and a decrease in the latter). There is a positive correlation between these parameters ($r_s = 0.9$ ($P < 0.05$)). There is a simultaneous decrease in the portion of cells in metaphase, anaphase, and telophase in the area with high pollution which is associated with the parameter "mitotic index calculated excluding prophase cells" which considering the level of mitotic pathologies indicates considerable changes in the genetic material of maternal birch plants and their seed progeny (plantlets) as compared to those in the area with low pollution.

An increase in the number of cells with persistent nucleoli in the root meristem cells of plantlets of the weeping birch at the metaphase, anaphase, and telophase stages of mitosis in the experiment areas as compared to the control group might be explained by the influence of anthropogenic pollution on the studied objects.

Persistent nucleoli look like round or drop-shaped structures connected with the chromosomes at metaphase, anaphase, and early telophase of mitosis when the nuclear membrane is absent, and chromosomes are significantly reduced. The phenomenon of the appearance of the persistent nucleoli at such mitosis phases as metaphase, anaphase and

early telophase (Sokolov *et al.*, 1974) is not typical for the normal course of division, because, as a rule, the nucleolus disappears at prophase and recovers only at late telophase (Butorina and Isakov, 1989). According to A. K. Butorina *et al.* (Butorina *et al.*, 1997; Vostrikova and Butorina, 2006), persistent nucleoli at metaphase, anaphase, and telophase of mitosis can be called an individual case of the nucleolar activity enhancement, an adaptive mechanism for exposure to the unfavorable environment. An increase in the number of cells with persistent nucleoli indicates an enhanced nucleolar activity due to the additional synthetic activity of ribosomal genes, similar to the synthesis of "heat shock" proteins. The anthropogenic impact on maternal plants can be compared to the influence of high temperature on living organisms which is stressful for them and leads to the synthesis of stress proteins, heat shock proteins, whose synthesis can be induced not only by heat shock but also by a number of other unfavourable impacts, including those of heavy metals (Chirkova, 2002).

The level of mitotic pathologies in the root meristem cells of plantlets from areas with high pollution considerably exceeds the control parameter ($P < 0.01$) and the same parameter for seed progeny for the area with low pollution (see Table 2; $P < 0.01$), although there is no difference in germination as compared to the control group (see Table 1). However, it was noticed that the mitotic index during the experiment was lower than the control (see Table 2), which means that germinating seeds, being living organisms, seek reserve growth capacities, i.e., mechanisms responsible for the adaptation to the environmental factors are triggered. At the cellular level, it results in an increased intensity of metabolic processes: an increase in the amount of total protein, nucleolar activity (an increased number of cells with persistent nucleoli at the metaphase, anaphase, and telophase of mitosis).

Therefore, it is possible that the quality of the seed material collected in the area with low pollution was lower than in the control area. However, it was higher than that in the area with high pollution. Due to a high level of mitotic pathologies in the root meristem cells of plantlets of the weeping birch from polluted areas, it is a possibility of genetic instability of maternal plants.

Therefore, it is not recommended to collect pharmacognosy material and seeds for planting material from the analyzed trees. However, they can be used for selection purposes as initial material for the production of

new types (as mutable samples) in artificial mutagenesis.

4. CONCLUSIONS:

Therefore, the parameter "amount of total protein" (protein level) can be called a seed germination marker that determines its germination abilities. Hence, the seed progeny of the weeping birch collected in the area with low pollution levels might have protein synthesis stimulation. These maternal trees and their progeny can be used as a source of biologically active substances and for the collection of plant material for pharmacognostic purposes, as well as a stable species to be used for the planting of urban areas.

This research showed that the amount of total protein (biochemical parameter) is associated with cytogenetic characteristics. A comprehensive study of cytogenetic and biochemical parameters can allow a quick and adequate evaluation of the seed quality of unknown origin which is especially important for plant industry and forestry as it allows selecting stable planting material and for the collection of material for pharmacognostic purposes in areas with different ecological pressure. This research indicates a possibility of using cytogenetic and biochemical parameters to characterize the ecological state of environment, on the one hand, and, on the other hand, the state of maternal trees and their progeny. Determining cytogenetic indices and the amount of total protein allows forecasting the germination capacities and germination abilities of the seeds of the weeping birch.

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Table 1. Quantitative changes of the total protein and germination abilities of seeds of the weeping birch (*Betula pendula*), collected from trees growing in different ecological environments

Tree number	Total protein in seeds, mg/ml	Total protein in plantlets, mg/ml	Germination, %
Botanical Garden (control)			
1	0.168±0.03	0.155±0.03	28
2	0.182±0.02*	0.160±0.02	34
3	0.136±0.01	0.148±0.02	25
4	0.580±0.01**	0.208±0.03*	62
5	0.460±0.06**	0.176±0.03	55
average	0.305±0.038	0.170±0.02	40.8
Platonov street (area with a low pollution level)			
1	0.730±0.03*	0.164±0.03	82
2	0.952±0.05*	0.136±0.02	94
3	0.606±0.09	0.286±0.04*	63
4	0.726±0.07*	0.260±0.03*	75
5	0.624±0.05	0.225±0.04**	68
average	0.728±0.035 ^b	0.214±0.03 ^a	76.4 ^b
Leninsky Avenue (area with a high pollution level)			
1	0.350±0.02**	0.144±0.04*	28
2	0.120±0.01	0.160±0.03*	12
3	0.380±0.04**	0.135±0.02	33
4	0.436±0.03**	0.122±0.03	44
5	0.558±0.02**	0.118±0.02	51
average	0.369±0.031 ^c	0.136±0.03 ^c	33.6

Note: * differences between trees of the same sample are reliable ($P < 0.05$)

** differences between trees of the same sample are reliable ($P < 0.01$)

a differences with the control are reliable ($P < 0.01$)

b differences with the control are reliable ($P < 0.001$)

c – differences with the area with low pollution are reliable ($P < 0.001$).

Table 2. Cytogenetic parameters ($\bar{x} \pm S\bar{x}$) of seed progeny of *Betula pendula* in different areas of Voronezh

P.	MI without P, %	MI, %	PM, %	PN, %	P, %	M, %	A, %	T, %
Botanical Garden (control)								
av	2.9±0.07	4.0±0.09	3.6±0.5	13.8±1.1	28.1±0.4	31.0±1.2	11.5±0.4	25.8±0.9
Platonov street (area with a low pollution level)								
av	2.1±0.1*	3.3±0.2**	13.5±1.3 **	19.5±1.2*	35.7±1.1* **	34.5±1.3	9.3±0.7	20.6±1.2
Leninsky Avenue (area with a high pollution level)								
av	1.8±0.1***	3.5±0.2*	22.3±1.1 ** b	17.2±0.7*	50.0±0.8* ** c	23.7±1.1* a	8.0±0.8**	18.9±1.0* *

Note: P. – parameters av – average value for the studied area; MI, % – mitotic index calculated including cells at the prophase stage; MI without P, % – mitotic index calculated excluding prophase cells; MP, % – level of mitotic pathologies; PN, % – level of cells with persistent nucleoli; P, % – number of cells at the prophase stage; M, % – number of cells at the metaphase stage; A, % – number of cells at the anaphase stage; T, % – number of cells at the telophase stage.

* differences with the control are reliable ($P < 0.05$)

** differences with the control are reliable ($P < 0.01$)

*** differences with the control are reliable ($P < 0.01$)

^a differences with the area with low pollution are reliable ($P < 0.005$).

^b differences with the area with low pollution are reliable ($P < 0.01$).

^c differences with the area with low pollution are reliable ($P < 0.001$).